Distinction Of The Present Invention From The Prior Art

Applicants have discovered that ubiquitin fused to an epitope or epitopes in a defined manner, is useful for the stimulation of a highly specific immune response when administered to an animal. This immune response is highly specific for the fused epitope(s) (even if the epitope is otherwise non-immunogenic), and generates little to no immune response to the ubiquitin portion of the fusion protein. In the present invention, ubiquitin is used as a scaffold to stabilize and display recombinant heterologous epitopes in the generation of an immune response. This use necessitates that the fused epitopes not be susceptible to cleavage by ubiquitin specific proteases, which are abundantly present in animal tissue and would readily cleave the epitope from ubiquitin, eliminating the conferred immunogenicity. Prior to Applicants' invention, ubiquitin fusion proteins were used to either study the ubiquitin degradation pathway of proteins, or to stabilize and promote proper folding of a fused protein during synthesis/purification in a bacterial system. The ubiquitin fusion proteins generated in the prior art for stabilization of a fused ubiquitin could be readily cleaved from the fused protein, either experimentally by exposure to ubiquitin specific proteases, or *in vivo* (e.g., upon injection into an animal) where ubiquitin specific proteases are abundant. This susceptibility was viewed as a positive attribute of the fusion proteins of the prior art.

Claims 2-12 and 14-19 specifically describe a unique permutation of a ubiquitin fusion protein which is generated for the newly discovered use of ubiquitin as an immunogenic carrier: a ubiquitin fusion protein which contains ubiquitin fused to two or more identical epitopes, which is <u>not</u> susceptible to cleavage by ubiquitin specific proteases. The presence of multiple copies of the epitope increases the immunogenicity. The resistance to cleavage is necessary for those epitopes to remain attached to ubiquitin when injected into an animal. These properties are encompassed in the limitations of Claim 2, the relevant

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limitations being that it a) contains two or more identical epitopes fused to ubiquitin and b) that the fused epitopes are not cleavable from the ubiquitin fusion protein by exposure to ubiquitin-specific proteases. Because it was not previously recognized that ubiquitin could be used, in the form of a fusion protein, as an immunogenic carrier of an otherwise non-immunogenic epitope, there was no prior motivation to produce a ubiquitin fusion protein which met all of these limitations.

Amendment of the Claims

Claim 2 has been amended to specifically recite that "the epitope-containing segment is not cleaved from the ubiquitin fusion protein by exposure to ubiquitin-specific proteases." Support for the use of this term is found on page 14, line 24-33 of the Specification which states:

> As is well known to those of skill in the art, ubiquitin can be made resistant to ubiquitin-specific proteases by altering residues at the C-terminus of ubiquitin. For example, by altering the identity of the amino acid at position 76 of ubiquitin (e.g., from glycine to valine or cysteine), the rate of cleavage of a C-terminal ubiquitin fusion can be substantially reduced to the point where cleavage can not be detected using the assays typically employed for monitoring such cleavage.

The term "not cleaved" as used in the claims refers to a ubiquitin fusion protein which, upon exposure to ubiquitin specific proteases, is not detectably cleaved from its epitope containing segment, using standard assays of detection. As indicated in the above quoted passage.

Rejections Under 35, USC, 112, Second Paragraph

Claims 2-8, and 14-19 have been rejected under 35 USC 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. More specifically, the Patent Office objects to the term "efficiently cleaved" used in

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Claim 2, as unclear. This rejection has been obviated by amendment of Claim 2 to eliminate the term "efficiently cleaved" and instead recite:

> "... the epitope-containing segment is not [efficiently] cleaved from the ubiquitin fusion protein by exposure to ubiquitin-specific proteases ..."

The use of the term "not cleaved" is thought sufficiently clear.

Rejection Under 35 USC 102

Claims 2, 3, 8, 13, 21, 22, 27, 33 and 75 remain rejected under 35 USC 102(e) as being anticipated by Rechsteiner et al. More specifically the Patent Office states:

> Applicants argue that this rejection has been obviated by the amendment of Claim 2 to include the limitation that the epitope containing segment is not efficiently cleaved from the ubiquitin fusion protein by exposure to ubiquitin-specific proteases in vivo. ... Rechsteiner et al. disclose synthesis and recovery of ubiquitin-carboxyl extension peptides wherein the peptides contain two to forty amino acid residues, and wherein the peptides can be recovered as ubiquitin fused extension products (Ub-CTEP) or alternatively can be cleaved from the ubiquitin by an appropriate peptidase. Thus the alternative embodiment where the peptide is recovered still fused to ubiquitin, anticipated amended claims 2-3, 8, 13, and 75.

This rejection of Claims 21, 22, 27, 33 and 75 has been obviated by cancellation of these claims. This rejection of Claims 2, 3, 8, and 13, is respectfully traversed. With respect to Claims 2, 3, and 8, the ubiquitin-fusion protein of Rechsteiner et al. has different physical characteristics than the fusion protein of the present invention in that it is susceptible to cleavage by ubiquitin specific proteases. The fusion protein of Claim 2, 3, and 8 is resistant to cleavage by ubiquitin specific proteases, and this resistance results from structural differences in the respective fusion proteins. Specifically, this resistance is due to the location of the fusion protein within the molecule, and alternatively the amino

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acid sequences of the ubiquitin molecule when the fusion is made at the C-terminus. This characteristic difference between the fusion protein of Rechsteiner et al. and the ubiquitin fusion protein of Claim 2, 3 and 8 carries patentable weight which must be fully considered by the Patent Office in determining patentability. The fact that the ubiquitin fusion protein of Rechsteiner et al. can exist in an uncleaved form (e.g., when synthesized in *E. coli*) is irrelevant since the fusion protein would be rapidly and efficiently cleaved upon exposure to ubiquitin-specific proteases (e.g., upon administered to a mammal as an immunogen).

Claims 2-3, 8, 13, 21, 22, 27, 33 and 75 have been rejected under 35 USC 102(b) as being anticipated by Wittliff et al. This rejection of Claim 13, 21, 22, 27, 33 and 75 has been obviated by cancellation of these claims. This rejection of Claims 2, 3, and 8 is respectfully traversed. It is respectfully submitted that the previous traversal of this rejection of Claims 2-3, and 8, arguing that the fusion protein of Wittliff et al. does not contain two or more identical epitopes, was not fully considered in that the argument is not addressed in the present Office Action. With respect to Applicants' previous arguments, the Patent Office states:

> These arguments are not persuasive, because the hFSHR inherently comprises more than one epitope, some of which are not contiguous.

This does not discuss the limitation that the fusion protein of the present invention contains two or more identical epitopes, present in Claims 2, 3, and 8. Applicants maintain the previously stated traversal of this rejection: Claims 2-3, and 8 specifically recite "A ubiquitin fusion protein comprising ubiquitin fused to a single epitope-containing segment, the epitope containing segment comprising two or more identical epitopes..." The identical epitopes of the present invention are indicated by the presence of repeating identical sequences of 10 or more amino acids (for example, see page 44, line 17-20, which

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refers to multiple copies of the GnRH epitope). Wittliff et al. teaches ubiquitin fused to an active human estrogen receptor. There are no such repeated sequences present in the human estrogen receptor which qualify as identical epitopes, and in the absence of this, there is no indication that the human estrogen receptor contains two or more identical epitopes. Therefore, Wittliff does not teach ubiquitin fused to two or more identical epitopes and does not anticipate Claims 2-3, 8.

Claims 2, 3, 8, 13, 21, 22, 27, 33, and 75 have been rejected under 35 USC 102(b) as being anticipated by Vannier et al. More specifically the Patent Office states:

> Applicants argue that Vannier et al. teach a fusion protein comprised of ubiquitin fused to the extracellular domain of amino acids 23-358 of human FSH receptor to the C-terminus of ubiquitin, thus this fusion protein does not contain two or more identical epitopes or two or more noncontiguous epitopes. This argument is not persuasive, because the hFSHR inherently comprises more than one epitope and some of these epitopes are not contiguous.

This rejection of Claims 13, 21, 22, 27, 33, and 75 has been obviated by cancellation of the claims. The rejection of Claims 2, 3, and 8 is respectfully traversed. The fusion protein taught by Vannier et al. contains amino acids 23-358 of hFSHR fused to the C-terminus of ubiquitin, and shares the same relevant characteristics as the fusion protein disclosed by Wittliff et al., discussed above. Again, there is no indication in the Office Action that Applicants' argument with respect to the patentable distinction of Claims 2, 3, and 8 over Vannier et al. were considered. To reiterate the argument, Claims 2, 3 and 8 specifically recite "A ubiquitin fusion protein comprising ubiquitin fused to a single epitope-containing segment, the epitope containing segment comprising two or more identical epitopes..." Vannier et al. teaches ubiquitin fused to amino acids 23-358 of FSHR, at the C-terminus. There are no repeated sequences in the FSHR which qualify as identical epitopes. Therefore, Vannier et al. does not teach ubiquitin fused to two

or more identical epitopes and does not anticipate Claims 2-3, 8. The presence of multiple epitopes in the FSHR sequences of the Vannier et al. fusion protein does not denote the presence of identical epitopes. As stated in the MPEP:

> "In relying on the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art." *Ex parte Levy*, 17 USPQ2d 1461, 1464

The examiner has not supplied any such basis in fact or technical reasoning.

Rejection Under 35 USC 103(a)

The rejection of Claims 15, 35, 55, 72, 81, and 83 made under 35 USC 103(a) as being unpatentable over van der Zee et al. in view of Vannier et al. has been maintained. In response to Applicants' arguments regarding this rejection, the Patent Office states:

> Applicants argue that the ubiquitin fusion protein of the present invention provides the double advantage of conferring antigenicity to a relatively small peptide sequence, which allows specific targeting of the immunogenic response to one or two epitopes of a protein and generating minimal if any immune response to the ubiquitin carrier, and that large peptides/protein are often immunogenic in the absence of a carrier, while small peptides are not. Applicants also argue that the ability of ubiquitin to function well as a carrier for the epitopes specified by the applicant's invention could not have been predicted by one of skill in the art with any degree of certainty, because ubiquitin is highly conserved protein and thus is minimally antigenic itself ....
>
> **With respect to Applicants (first) argument, Applicants are arguing limitations that are not in the claims.** The size of the peptide fused to ubiquitin is irrelevant to the instant claims. Also, since Vannier et al. was able to elicit an immune response against FSHR fused to ubiquitin, it would be obvious to the skilled artisan to fuse small peptides to ubiquitin with great expectation of success to elicit an immune

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> response against said small peptides, because there is nothing in the Vannier et al. reference that suggests, that small peptides fused to ubiquitin may not elicit an immmune response. (emphasis added)

This rejection of Claims 35, 55, 72, 81, and 83 has been obviated by cancellation of the claims. This rejection of Claim 15 is respectfully traversed. With respect to the above quoted statement that the size of the peptide fused to the ubiquitin is irrelevant to the instant claims, Applicants respectfully point out that the size of the peptide is relevant with respect to the cited prior art of Vannier et al. and van der Zee et al., the combined teachings of which the Patent Office states would have motivated one of skill in the art to generate the ubiquitin fusion protein specifically described in Claim 15. The relevant argument for patentability of the claims over the cited prior art is that the ability of ubiquitin to function well as an immunogenic carrier for the epitopes could not have been predicted by one of skill in the art with any degree of certainty, because ubiquitin is such a highly conserved protein and thus is minimally antigenic itself. The relevant limitations to the claims with respect to this argument, stated in Claim 2, is that a) ubiquitin is fused to an epitope containing segment comprising two or more identical epitopes, and b) that "the ubiquitin fusion protein has the ability to stimulate an immune response to the heterologous epitope contained therein." Prior to the present invention, one of ordinary skill in the art would have predicted that a ubiquitin fusion protein consisting of ubiquitin and the non-immunogenic GnRH decapeptide (the GnRH decapeptide is specifically described as non-immunogenic in the first paragraph of van der Zee et al.) would not have the ability to stimulate an immune response to the heterologous epitope contained therein, specifically because of the minimal antigenicity of ubiquitin. One of skill in the art would not have been motivated by the combined teachings of Vannier et al. and van der Zee et al. to generate a ubiquitin fusion protein

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fused to two or more identical epitopes in the absence of expectation of immunogenicity for said epitopes.

The Patent Office further states:

> With respect to Applicants second argument, Vannier et al. was able to elicit an immune response against the Ub-FSHR protein, showing that ubiquitin functions well as a carrier.

Again, this finding of Vannier et al. does not show that ubiquitin functions as an immunogenic carrier because the Ub-FSHR protein is susceptible to cleavage by ubiquitin specific proteases *in vivo*, and thus is rapidly cleaved upon injection by ubiquitin-specific proteases. The fact that an immune response was elicited to the (cleaved) C-terminus is due to its intrinsic immunogenicity, and not to its fusion to ubiquitin.

Claims 4-7, 14-19, 21-26, 33-36, 38-40, 44-47, 54-56, 71-73 and 81-83 have been rejected under 35 USC 103(a) as unpatentable over Wittliff et al. in view of van der Zee et al. More specifically the Patent Office stated previously:

> it would have been obvious to modify the GnRH fusion protein taught by van der Zee, by generating GnRH as a ubiquitin fusion protein, using the teachings of Wittliff et al. because Wittliff et al. teach that the expression of proteins as ubiquitin fusions facilitates stabilization and increases efficiency of translation, and the attachment of ubiquitin promotes proper folding, thus preserving the protein's biological activity ...

In the present Office Action, the Patent Office states that Applicants' previous arguments traversing this rejection are not persuasive because:

> Wittliff et al. demonstrated that the UB-Er fusion behaved identically as the wild type receptor and is recognized by monoclonal antibodies against two epitopes of the human estrogen receptor, thus showing that ubiquitin functioned well as carrier and that an immune response was not elicited against it.

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This rejection of Claims 13, 21-26, 33-36, 38-40, 44-47, 54-56, 71-73 and 81-83 has been obviated by cancellation of these claims. This rejection of the remaining claims is respectfully traversed. Preliminarily, Applicants point out that the reasoning in the above quoted statement is flawed. The fact that monoclonal antibodies generated against human estrogen receptor (note they were not generated against the ubiquitin-ER fusion protein) recognize the UB-ER fusion protein does not indicate that the UB-ER fusion protein is immunogenic for the estrogen receptor. It is well known in the art that antibody recognition of a polypeptide in one context (e.g., in the context of a fusion protein), and the ability to generate an immune response to that polypeptide in the same context are two different phenomena, the former of which does not necessarily indicate the latter. Furthermore, the ability of monoclonal antibodies specific for the estrogen receptor to recognize the UB-ER fusion protein does not indicate whether or not an immune response is elicited against the ubiquitin portion of the fusion protein. The fact that antibodies generated to ubiquitin were also found to recognize the UB-ER fusion (Wittliff et al., page 22019, column 1, paragraph 2, last sentence), further indicates the inaccuracy of this line of reasoning.

To restate Applicants' argument traversing this rejection, Wittliff et al. disclose a ubiquitin fusion protein fused to the complete amino acid sequence of the human estrogen receptor (described as a 40-45 kD protein, see page 22019, third paragraph, second column) at the C-terminus of ubiquitin. This fusion protein is cleavable, and is not shown to be antigenic for the human estrogen receptor epitopes. van der Zee et al. teaches the fusion of the short GnRH decapeptide to the highly immunogenic carrier P-fimbriae. The short GnRH decapeptide is specifically described as non-immunogenic in mammals (see first paragraph of van der Zee et al.). One of skill in the art would not have been motivated to combine the teachings of Wittliff et al. with the teachings of van der Zee et al. to produce the

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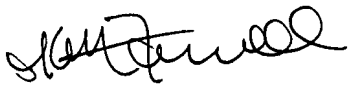
ubiquitin fusion protein of the present invention given the prevailing expectation in the art that the ubiquitin fusion protein which resulted would be non-immunogenic for fused epitopes which themselves were not immunogenic.

Summary

In light of the above amendment and remarks, reconsideration of the subject patent application is respectfully requested.

Respectfully submitted,



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